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Differential Neural Responses Evoked by Orthonasal versus Retronasal Odorant Perception in Humans

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Summary

Odors perceived through the mouth (retronasally) as flavor are referred to the oral cavity, whereas odors perceived through the nose (orthonasally) are referred to the external world. We delivered vaporized odorants via the orthonasal and retronasal routes and measured brain response with fMRI. Comparison of retronasal versus orthonasal delivery produced preferential activity in the mouth area at the base of the central sulcus, possibly reflecting olfactory referral to the mouth, associated with retronasal olfaction. Routes of delivery produced differential activation in the insula/operculum, thalamus, hippocampus, amygdala, and caudolateral orbitofrontal cortex in orthonasal > retronasal and in the perigenual cingulate and medial orbitofrontal cortex in retronasal > orthonasal in response to chocolate, but not lavender, butanol, or farnesol, so that an interaction of route and odorant may be inferred. These findings demonstrate differential neural recruitment depending upon the route of odorant administration and suggest that its effect is influenced by whether an odorant represents a food.

Introduction

An odor molecule may reach the olfactory epithelium via the nose (orthonasal olfaction) or the mouth (retronasal olfaction) (Figure 1). When an odor is sensed orthonasally, it is perceived as originating from the external world. In contrast, when an odor is sensed retronasally, it is perceived as arising from the mouth (Murphy et al., 1977; Rozin, 1982). The illusion that retronasally perceived odors are localized to the mouth is so powerful that people routinely mistake retronasal olfaction for "taste" (Murphy et al., 1977; Rozin, 1982). For example, we may say that we like the "taste" of a wine because of its fruity or spicy notes. However, gustation refers only to the sensations of sweet, sour, salty, savory, and bitter, and thus the pleasant "taste" to which we refer is actually a pleasant odor sensed retronasally.

A simple experiment to illustrate this illusion is to pinch the nose while eating or drinking. This disruption of airflow stops odor molecules from traversing the nasopharynx and blocks flavor perception. When the nose is released, and retronasal olfaction is resumed, the flavor is immediately localized to the mouth. The fact that the olfactory referral illusion is maintained even though the subject is now aware that the experience is related to an event in the nose demonstrates that olfactory referral is robust and cognitively impenetrable.

In 1982, Rozin observed that "olfaction is the only dual sensory modality, in that it senses both objects in the external world and objects in the body (mouth)" and thus proposed that "the same olfactory stimulation may be perceived and evaluated in two qualitatively different ways depending on whether it is referred to the mouth or the external world" (Rozin, 1982). Studies testing Rozin's hypothesis have yielded mixed results, with several authors concluding that retronasal and orthonasal olfaction differ only in the efficiency with which odors are delivered to the olfactory epithelium (Pierce and Halpern, 1996; Voirol and Dagnet, 1986). However, most studies comparing ortho- versus retronasal olfaction have focused upon qualities of the experience that provide information about the quantity or identity of the sensory stimulus, whereas the key distinction Rozin had made was that route of delivery influenced not *what* the stimulus was but rather *where* the stimulus was perceived and what this in turn implied about the nature of the stimulus. Although several studies have examined brain responses to retronasal olfactory stimulation (Cerf-Ducastel and Murphy, 2001; de Araujo et al., 2003; Small et al., 2004), none have directly compared orthonasal and retronasal stimulation in the same subjects or considered the possibility that the effects of route of stimulation depend on the way that odors are typically sensed. For example, food odors are normally experienced both orthonasally and retronasally, whereas nonfood odors are perceived only orthonasally. Therefore, it is possible that the route of stimulation may have different effects for food versus nonfood odors. We also reasoned that physiochemical aspects of an odorant, such as its lipophilicity, might differentially influence neural activity evoked by different routes of administration.

Finally, previous studies comparing orthonasal and retronasal olfaction achieved retronasal olfaction by presenting liquids and orthonasal olfaction by presenting vapors (Cerf-Ducastel and Murphy, 2001; de Araujo et al., 2003; Small et al., 2004), making it impossible to ascertain whether the differential activation is related to route of delivery or to the physical differences in the stimuli and the different somatosensory sensa-

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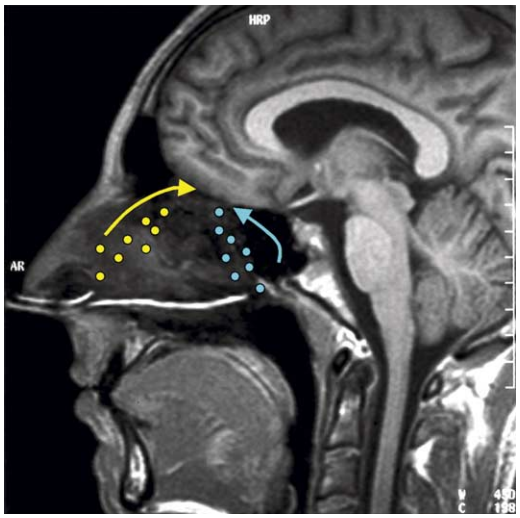

Figure 1. An MRI Image Showing Placement of the Nasal Cannulae at the External Nares, to Achieve Orthonasal Delivery, and at the Retropharynx, to Achieve Retronasal Delivery

Odorized air was administered through these cannulae in this experiment. All insertions were performed under endoscopic guidance (for details of the stimulation technique see Heilmann and Hummel, 2004) (white lines in cross-section). Yellow dots and arrow depict the idealized distribution and flow direction of odorants delivered orthonasally, and turquoise dots and arrow represent the distribution and flow direction of odorants delivered retronasally.

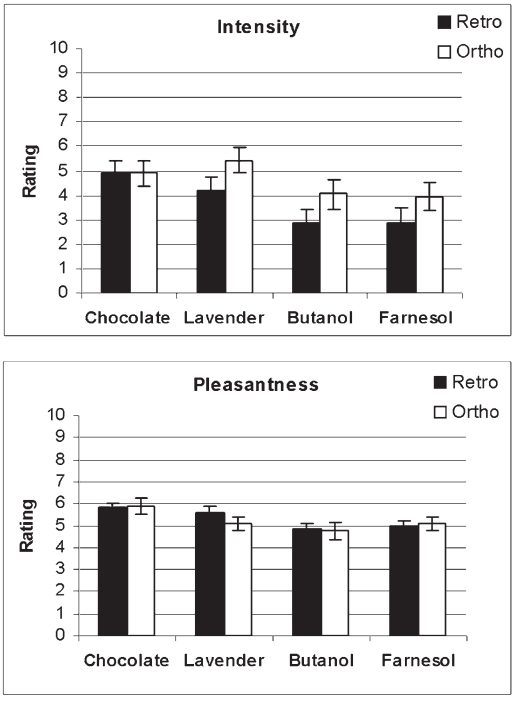

Figure 2. Mean Ratings of Odor Intensity and Pleasantness

Error bars represent the standard error of the mean.

tions that they evoke. To circumvent this problem in the current study, we employed a technique of odorant delivery in which odors can be delivered as vapors via both ortho- and retronasal routes (Heilmann and Hummel, 2001). This is achieved by inserting tubes into the nose under endoscopic guidance so that one tube ends at the external nares and the other tube ends at the nasopharynx (Figure 1). Importantly, using this method, the perception is maintained that orthonasally delivered odors arise from the nose (and thus come from the external world), whereas retronasally perceived odors arise from the mouth (Hummel et al., 2005). Moreover, the localizability cannot be attributed to detection of airflow differences because a constant stream of airflow is maintained through both tubes at all times (Heilmann and Hummel, 2004). Our goal was to use this method in conjunction with fMRI to determine whether different routes of odorant delivery would produce differential neural activation, and whether this differential activation would vary with either the physiochemical properties of the odor or odor type (i.e., food or nonfood odor). Chocolate odor was selected as the food odor because the brain response to chocolate has been previously elucidated (Small et al., 2001). Lavender odor was chosen because it is a nonfood odor that has a similar quality of pleasantness as the chocolate odor. Butanol and farnesol were selected because of their physiochemical properties, with butanol being more hydrophilic and farnesol being more lipophilic.

Results

The study conformed to a two-factorial design with "odor" (lavender, butanol, farnesol, and chocolate) and "mode of delivery" (orthonasal and retronasal) representing the two within-subjects factors. This resulted in eight odor conditions (CR = chocolate retro; CO = chocolate ortho; LR = lavender retro; LO = lavender ortho; BR = butanol retro; BO = butanol ortho; FR = farnesol retro; and FO = farnesol ortho), each with its own odorless baseline condition. Eleven healthy right-handed subjects were scanned.

Perceptual Ratings of the Odorants

Subjects provided ratings of stimulus pleasantness and intensity after each run, using an 11-point category scale (10 = extremely strong/extremely pleasant; 5 = moderate/neutral; and zero = odorless/extremely unpleasant). Ratings were entered into a 2 × 2 × 2 repeated measures ANOVA with odorant, rating, and mode of delivery as within-subject variables. Mean ratings are presented in Figure 2. There were no main effects of odorant {$F_{(1,11)}$ 1.2; $p = 0.3$} or mode of delivery {$F_{(1,11)}$ 2.0; $p = 0.18$}. However, a significant 3-way interaction occurred between mode of delivery, odorant, and rating {$F_{(2,11)}$,6.4; $p = 0.02$}, such that the orthonasally presented lavender was rated significantly stronger than the retronasally presented lavender ($p = 0.005$). No other significant differences in the ratings were found. Because visual inspection of the data suggested that orthonasal perception of lavender, farnesol, and butanol might be more intense than retronasal perception of these odors, a separate ANOVA was conducted on the data from these three odorants. An effect of route of delivery on intensity perception {$F_{(1,11)}$,19; $p = 0.001$} confirmed our suspicion. By contrast, a Student's t test comparing intensity perception of retrona-

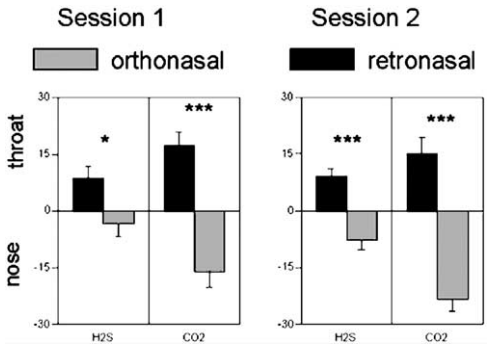

Figure 3. Odorant Localization

Preliminary data from 20 subjects, showing that they perceive the orthonasal odor as coming from the front of the nasal cavity and the retronasal odor as coming from the back of the nasal/oral cavity. This is despite the fact that constant airflow is maintained through both routes at all times and that there is no change in air pressure or flow rate when switching between odor and no odor (Kobal, 1981). One odor was a specific olfactory stimulant (hydrogen sulfide, H2S), and the other had a significant trigeminal component (carbon dioxide, CO2). Results represent the mean rating from 20 subjects. Error bars represent the standard error of the mean. Positive numbers indicate that subjects perceived the odor at the back of the nasal/oral cavity (pharynx near the throat area), and negative numbers indicate that subjects perceived the odor at the front of the nose; the higher the numbers, the more certain were subjects about their decision (scale range, –50 to 0, and 0 to 50). Data were obtained during two sessions separated by at least 1 day. Stimuli were presented for 200 ms using (birhinal olfactometer OM6b, Burghart Instruments, Wedel, Germany). Thus, stimulation was the same as that used in the fMRI study. (Student's t test: *, $p < 0.05$; ***, $p < 0.001$).

sal versus orthonasal chocolate revealed no differences: $t = 0.86$; $p = 0.93$.

Participants were questioned about the perceived location of the odorants. In agreement with previous findings (Hummel et al., 2005; Rozin, 1982; Murphy et al., 1977; and see Figure 3), the retronasally presented odors were perceived as arising from the oral cavity (back of the throat), whereas the orthonasally presented odors were perceived as coming from the tip of the nose. In contrast, and also consistent with previous work in our lab (manuscript in preparation), subjects were not able to distinguish presentation of odorless air via the two routes (performing at chance). A subsequent test with different subjects revealed that although subjects could distinguish orthonasal versus retronasal presentation of the chocolate odor (100%), they could not distinguish presentation to the left versus that to the right nostril (40 trials with average correct responses = 20.2 across six subjects). This indicates that the odor does not have a trigeminal component and argues against trigeminal cues facilitating orthonasal versus retronasal odorant localization.

Neuroimaging Data

Neuroimaging data were pre- and postprocessed with SPM2. Effects were thresholded at $p < 0.001$ uncorrected with a cluster criterion of three voxels. Figure 4 presents BOLD detectability maps demonstrating that we are able to measure signal from the orbitofrontal cortex (OFC) and amygdala (Parrish et al., 2000). BOLD

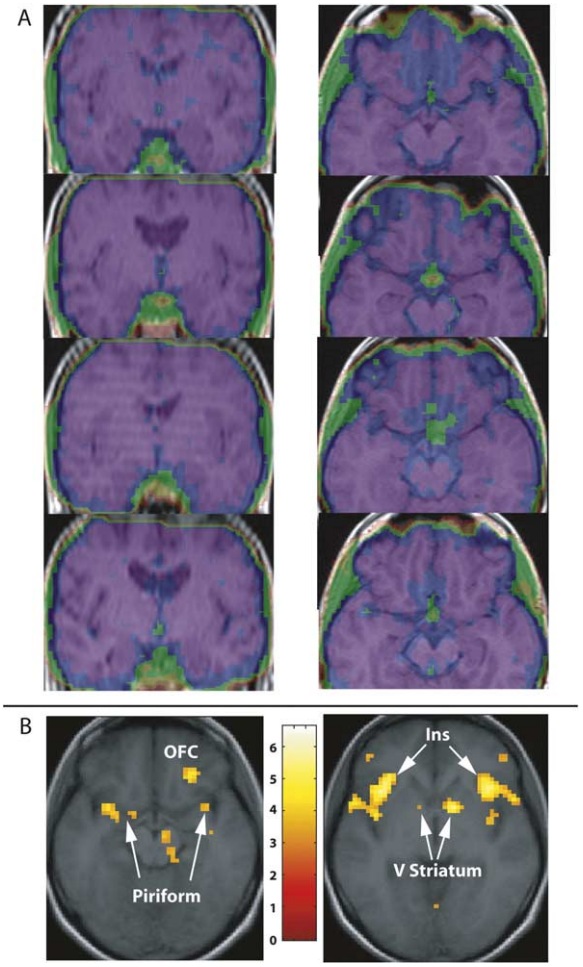

Figure 4. BOLD Detectability Maps and Main Effect of Odors

(A) BOLD detectability maps generated from four subjects, showing the ability to detect a greater than 0.5% signal change throughout most of the OFC and the amygdala. Purple indicates the ability to detect $\geq$ 0.5% signal change; blue, $\geq$1%; green $\geq$2%; yellow $\geq$4% signal change given the number of trials collected and α and $\beta = 0.05$. Each BOLD detectability map is superimposed upon the subject's T1-weighted MRI scan.

(B) Results from group random effects analysis of all odors—all odorless conditions. The t map is thresholded at $p < 0.001$, with a cluster threshold of $K < 3$. The color bar represents t values. Activation was observed in the orbitofrontal cortex (OFC), piriform cortex, insular cortex (Ins) and surrounding operculum, and ventral striatum (V Striatum). These results indicate that our odors and methods effectively activated the olfactory system.

detectability maps use data collected during an entire run (e.g., here, 5 min of data acquisition) to estimate the average percent signal change needed in order to detect a change (Parrish et al., 2000).

To determine the main effect of odorant, the eight odorant conditions were summed and contrasted with the eight odorless baseline conditions. In this confirmatory analysis, peaks are reported that survive a threshold of $p < 0.05$ corrected across the entire brain or $p < 0.001$ uncorrected if in predicted regions. As predicted, bilateral activity was observed in the piriform cortex (–39, 6, –12; $z = 4.7$; –15, 3, –18; $z = 3.5$; –30, 3, –21; $z = 3.8$; 36, 9, –15; $z = 4.6$), which represents the primary

olfactory region (Zatorre et al., 1992), the anterior insula/operculum (–39, 18, 0; z = 4.9; –60, 12, 3; z = 5.6; 33, 21, 0; z = 5.6; 39, 6, –12; z = 4.8), and ventral striatum (–9, 6, 3; z = 3.9 and 9, 6, 0; z = 5.9) (Figure 4). Activity was also observed in the right orbitofrontal cortex (OFC) (27, 33, –18; z = 4.4), which represents the secondary olfactory region (Zatorre et al., 1992), and in the left amygdala (–27, 0, –21; z = 4.3).

For the remaining analyses, the odorless conditions were first subtracted from their respective odorized conditions, and the resulting eight contrasts for each of the 11 subjects were entered into an ANOVA to assess group effects. Specific contrasts of interest were then performed. Predicted and unpredicted peaks with a voxel-wise $p < 0.05$ FDR-corrected across the entire brain were considered significant. Additionally, small volume corrections (SVC) were defined using coordinates from previously published peaks, to determine the significance of predicted peaks. Peaks with $p < 0.05$ FDR-corrected across the small volume were considered significant. Predicted regions included the olfactory system (insula, piriform, orbitofrontal cortex) and regions previously implicated in representing food reward (striatum, pallidum, insula, amygdala, hypothalamus, medial prefrontal cortex [e.g., Berridge, 1996; Kelley and Berridge, 2002; Saper et al., 2002]). Specifically, we predicted that the piriform cortex may respond preferentially to the orthonasal odors, reflecting its role in olfaction and sniffing (Sobel et al., 1998; Zatorre et al., 1992), and the insula and frontal and parietal operculum would respond to retronasal odors, reflecting the importance of these regions in taste, flavor, and oral cavity representation (Boling et al., 2002; Cerf-Ducastel et al., 2001; de Araujo et al., 2003; Frey and Petrides, 1999; Kinomura et al., 1994; Kobayakawa et al., 1996, 1999; Small et al., 1999, 2003, 2004). Additionally, in a previous study we identified a large area of activation in the anterior cingulate cortex extending into the medial OFC and subcallosal region in response to the receipt of chocolate. We therefore predicted preferential response in the same areas to retronasally presented chocolate odor (Small et al., 2001). In contrast, since the amygdala did not respond to the receipt of chocolate in that study but does respond to stimuli predictive of food reward (e.g., Gottfried et al., 2003), we hypothesized greater activity in the amygdala in response to orthonasally presented chocolate odor.

Effect of Route of Administration
Collapsed across Odorant

The contrast of all orthonasally delivered odors versus all retronasally delivered odors (baselines subtracted) was performed to identify regions responding preferentially to orthonasal olfactory perception irrespective of odorant quality. Activity was observed in the left frontal operculum at –60, 18, 3; z = 3.7. Since this region was not predicted, it is not considered significant. When all retronasal contrasts were compared with all orthonasal contrasts, a significant peak was observed at the base of the central sulcus extending from the postcentral into the precentral gyrus at –51, –9, 33; z = 3.7. This peak corresponds to the region responsive to oral cavity somatosensory stimulation in humans (Boling et al., 2002; Pardo et al., 1997) and may reflect the fact that retronasal odors, but not orthonasal odors, are referred to the mouth (Figure 5). This activation was significant after correcting across a small volume, defined using the peak from the Boling study (–53, 11, 30) as the center of a 20 mm diameter sphere ($p = 0.04$). Inspection of the activity within this region in response to retronasal versus orthonasal perception for each odor revealed a significant difference for LR > LO (–51, –6, 33; z = 3.9; $p = 0.04$, after SVC), but not for BR > BO or FR > FO, even after relaxing the threshold criterion for significance in the creation of the t map from $p < 0.001$ to $p < 0.005$. A small nonsignificant peak was observed in CR > CO at –54, –9, 36; z = 3.3. This finding, although interesting, was not consistent across all odors, and since three of the four odors were perceived as differentially intense (see Figure 2), intensity differences cannot be ruled out as a confounding factor.

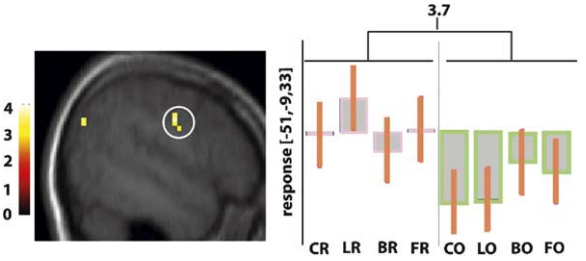

Figure 5. Result from the Analysis of Retro > Ortho, Collapsed across Odorant Type

The t map is thresholded at $p < 0.001$, with a cluster threshold of $K < 3$. The color bar represents t values. Activation at the base of the central sulcus is displayed in a sagittal section. The graph to the right shows responses (parameter estimates) to each of the eight odorant conditions minus each associated baseline condition at gyrus (coordinate defined on the y axis). Response is in arbitrary units. Pink lines represent confidence intervals. (CR = chocolate retro; LR = lavender retro; BR = butanol retro; FR = farnesol retro; CO = chocolate ortho; LO = lavender ortho; BO = butanol ortho; and FO = farnesol ortho).

Effects of Individual Odorants
Farnesol and Butanol

To determine the effect of physiochemical properties upon ortho- versus retronasal olfaction, we probed for differential activity as a function of route of delivery for farnesol and butanol. BO > BR, BR > BO, and FO > FR produced no differential brain activation (significant or nonsignificant) when the t map was thresholded at $p < 0.001$ and a cluster threshold of three voxels. Dropping the threshold to $p < 0.005$ yielded anterior cingulate activation in BR > BO (3, 33, 3; z = 3.0). Comparison of FR > FO also resulted in a peak in the anterior cingulate cortex (at –3, 27, 21; z = 3.8). Unfortunately, interpretation of this differential activation is complicated because subjects rated the orthonasal odor as more intense than the retronasal odor. No differential activation was observed by comparing BO + BR versus FO + FR (and vice versa), nor were differential responses observed for BO > FO, FO > FR, BR > FR, and FR > BR. Taken together, the results obtained under these spe-

cific conditions suggest that whether an odor is hydro- or lipophilic seems to play little role in the subsequent neural response.

Lavender

In addition to the activation at the base of the central sulcus, discussed above, comparison of LR > LO resulted in a significant peak in the ventral insula (at –42, –9, –6; z = 4.2; p = 0.01, following SVC). Two nonsignificant peaks were also observed: one in the left caudate nucleus (at –12, 24, –6; z = 4.1) and one in the left thalamus (at –15, –15, 0; z = 3.0). LO > LR yielded a peak in the right caudate nucleus (at 18, 12, 9; z = 4.5) that approached significance (p = 0.15, corrected across the entire brain), as well as some nonsignificant activity in extrastriate regions. These findings must also be interpreted with caution due to the differences in perceived intensity.

Chocolate

Chocolate was the only odor for which intensity and pleasantness ratings were equivalent for both orthonasal and retronasal perception. We therefore focused upon orthonasal versus retronasal comparisons of this odor. Comparison of CR > CO produced activation in the medial orbitofrontal cortex (or gyrus rectus, according to the terminology employed by Chiavaras and Petrides [2000]) at –3, 45, –18; z = 3.7; p = 0.02 after SVC, using a peak obtained in our previous study of brain response to chocolate (–1, 25, –19; [Small et al., 2001]) to define a 20 mm diameter sphere. Significant activity in the perigenual cingulate (3, 42, –9; z = 3.5; p = 0.02) was observed in the same small volume search. Nonpredicted peaks were observed in the superior temporal gyrus (57, –9, –6; z = 4.6; p = 0.07) and posterior cingulate cortex (–3, –27, 51; z = 4.3; p = 0.07), but are reported because they trended toward significance with use of the whole brain correction. In individual analyses, 10 of the 11 subjects showed activation in all of these regions.

Comparison of CO > CR produced activation in the thalamus (–3, 03, 15; z = 5.4; p = 0.004 and –6, 0, –3; z = 4.0; p = 0.04), right caudolateral orbitofrontal cortex (or lateral orbital gyrus [Chiavaras and Petrides, 2000]) (48, 36, –18; z = 4.4; p = 0.01), and right hippocampus (24, –18, –12; z = 3.9; p = 0.05). Additionally, several regions of perisylvian and insular cortex were activated preferentially in response to the orthonasally sensed chocolate odor, including the frontal operculum bilaterally (–57, 18, –3; z = 4.7; p = 0.007 and 54, 15, –3; z = 4.0; p = 0.04), temporal operculum/ventral insula bilaterally (–45, –9, –3; z = 3.8; p = 0.05 and 51, –12, 0; z = 3.4; p = 0.12), right supramarginal gyrus (66, –24, 15; z = 4.3; p 0.03), left anterodorsal insula (–36, 9, 3; z = 4.7; p = 0.007), and right far anterior insula (–30, 24, –3; z = 4.0; p = 0.04). Analyses of the individual data sets indicated that the thalamus and frontal and temporal opercula of all subjects were activated; in 10/11 the anterodorsal insula was activated and in 9/11 the OFC was activated.

These results clearly indicate that the neural response to an odor may be influenced by the route of administration, and thus support Rozin's conceptualization of olfaction as a dual sense modality. However, the magnitude of the effect was greatest for the chocolate odor, suggesting that differential neural recruitment may depend critically upon whether an odor has been previously experienced retronasally (i.e., whether it is a food odor). To further probe this possibility, we directly compared differential activation due to the route of administration for the chocolate odor with similar comparisons in the other three odors: {(CR > CO) versus (LR > LO + BR > BO + FR > FO)} and {(CO > CR) versus (LO > LR + BO > BR + FO > FR)}.

Comparison of CR > CO with the same subtraction for the lavender, farnesol, and butanol odors {(CR > CO) versus (LR > LO + BR > BO + FR > FO)} preferentially activated the perigenual cingulate region (6, 42, –9; z = 3.3; p = 0.04, SVC corrected) at the border between areas 32 and 25. This activity extended into the medial OFC (–3, 45, –18; z = 3.2; p = 0.04, SVC corrected). Activity in the superior temporal gyrus (at 57, –9, –6; z = 4.5) and in the posterior cingulate cortex (–6, –39, 39; z = 4.1) were also observed in this analysis. Thus, all four regions identified in CR > CO survived this direct comparison (Figure 6).

As reported above, analysis of the perceptual ratings indicated that lavender, farnesol, and butanol were perceived as more intense when delivered orthonasally than retronasally. In contrast, there was no difference in perceived intensity in orthonasal versus retronasal perception of the chocolate odor. Therefore, it is possible that an intensity effect could mask a route of administration effect for the nonfood odors. To verify that this was not the case, we regressed intensity ratings against neural response to the odors and searched for an intensity response in the four regions identified as responding selectively to CR (superior temporal gyrus, perigenual cingulate, medial OFC, and posterior cingulate cortex) by using the peak coordinate as a centroid for 15 mm diameter searches. There was no effect of intensity in any of the four regions during either orthonasal or retronasal stimulation. Therefore, it is unlikely that masking contributes to the selectivity of the differential response to the chocolate odor.

Comparison of CO > CR with the same contrast for the lavender, farnesol, and butanol odors produced activity in two regions of the thalamus (–3, –3, 15; z = 4.8; p = 0.01 and –6, 0, –6; z = 4.3; p = 0.03), the anterior dorsal insula (–36, 12, 0; z = 4.9; p = 0.01), and the temporal operculum/ventral insula (–45, –9, –3; z = 4.6 p = 0.02) that was significant with correction across the entire brain. Findings in the frontal opercula (–60, 12, 6; z = 3.8; p = 0.06 and 54, 15, –3; z = 3.7; p = 0.07) just missed significance with use of the whole-brain correction, and the hippocampal peak (at 21, –15, –15; z = 3.4) survived only when using a one-tailed SVC (p = 0.04). An additional peak was identified in the anterior ventral insula extending into the caudalmost OFC (at 39, 15, –18; z = 4.3; p = 0.03, corrected across the whole brain). The more anterior caudolateral OFC peak identified in CO > CR did not survive this analysis (Figure 7).

Finally, because the amygdala has been recently identified as a critical region involved in predictive coding of food reward in humans (Gottfried et al., 2003), we performed a region of interest (ROI) analysis in this area on the contrasts CO > CR masked exclusively by (LO > LR) + (BO > BR) + (FO > FR) and on the interaction {(CO > CR) versus (LO > LR + BO > BR + FO > FR)}. By

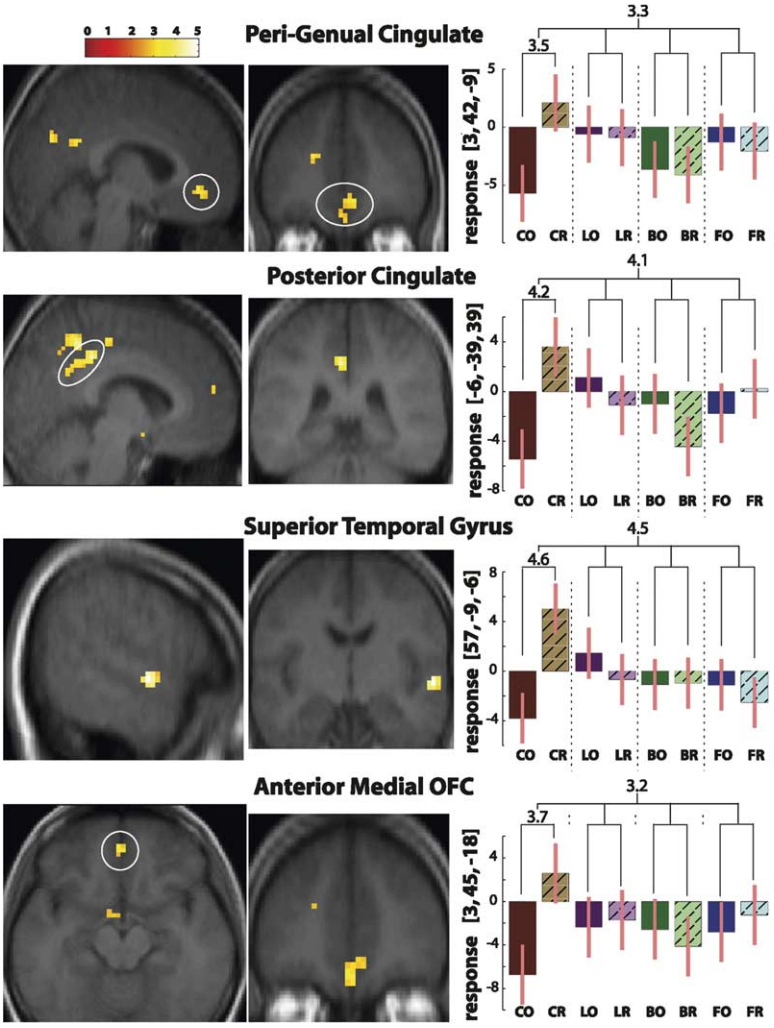

Figure 6. Interaction between Odorant Type and Route of Administration, Showing Regions Responding Preferentially to the Chocolate Odor Delivered Retronasally

Images from the analysis of {(CR > CO) versus (LR > LO + BR > BO + FR > FO)}. The t map is thresholded at $p < 0.001$ with a cluster threshold of $k < 3$. Graphs show response (parameter estimates) from each of the eight odorant conditions minus each associated baseline condition at the coordinate defined on the y axis. Response is in arbitrary units. Pink lines represent confidence intervals. (CR = chocolate retro; LR = lavender retro; BR = butanol retro; FR = farnesol retro; CO = chocolate ortho; LO = lavender ortho; BO = butanol ortho; and FO = farnesol ortho). Z values obtained in CR > CO are indicated by the lines adjoining these two conditions, and the z value for the interaction is indicated by the line joining the simple contrasts for the other odors.

using the exclusive mask in CO > CR, we filtered out all activity that was present in the masked contrasts, which enabled us to ensure that significant activation in the comparison of ortho- versus retronasal delivery was limited to the chocolate odor. The ROI was drawn with MRIcro (Rorden and Brett, 2000) and guided by the activation foci reported by Gottfried and colleagues. In their study they reported that a region extending from the posterior amygdala to the piriform cortex is sensitive to devaluation of visual stimuli predicting odors associated with food eaten to satiety (Gottfried et al., 2003). Several other studies of sensory cues predicting food reward also showed activity extending from the amygdala into the overlying piriform cortex (LaBar et al., 2001; O'Doherty et al., 2002). Therefore, our ROI originated in the piriform cortex (y = 6) and continued to the posterior amygdala (y = –12). These analyses yielded significant activation extending from the piriform cortex to the anterior region of the amygdala in the right hemisphere in CO > CR, masked exclusively by LO > LR + BO > BR + FO > FR (21, 0, –18; z = 3.1; 24, 3, –18; z = 3.1; 24, 6, –27; z = 3.3) (Figure 8), and in the interaction (at 21, –3, –30; z = 3.0; p = 0.05, corrected across the small volume and one-tailed).

Discussion

Rozin proposed that "the same olfactory stimulation may be perceived and evaluated in two qualitatively different ways depending on whether it is referred to the mouth or the external world" (Rozin, 1982). The current findings clearly support this notion by demonstrating that the neural response evoked by an odor may be influenced by its route of administration. First, we identified a region of the Rolandic operculum at the base of the central sulcus, corresponding to primary representation of the oral cavity (Boling et al., 2002; Pardo et al., 1997) that responded more to retronasal than to orthonasal olfactory stimulation, irrespective of odorant (Figure 5). This finding may reflect the fact that retronasally, but not orthonasally, delivered odors are perceived as originating from the oral cavity (Murphy et al., 1977). Because subjects could not distinguish between orthonasal and retronasal delivery of odorless air, the effect is unlikely related to differences in somatosensory stimulation. However, inspection of the individual contrasts revealed that activity in the Rolandic operculum was only observed in LR > LO and CR > CO, and for the latter, the findings only approached significance.

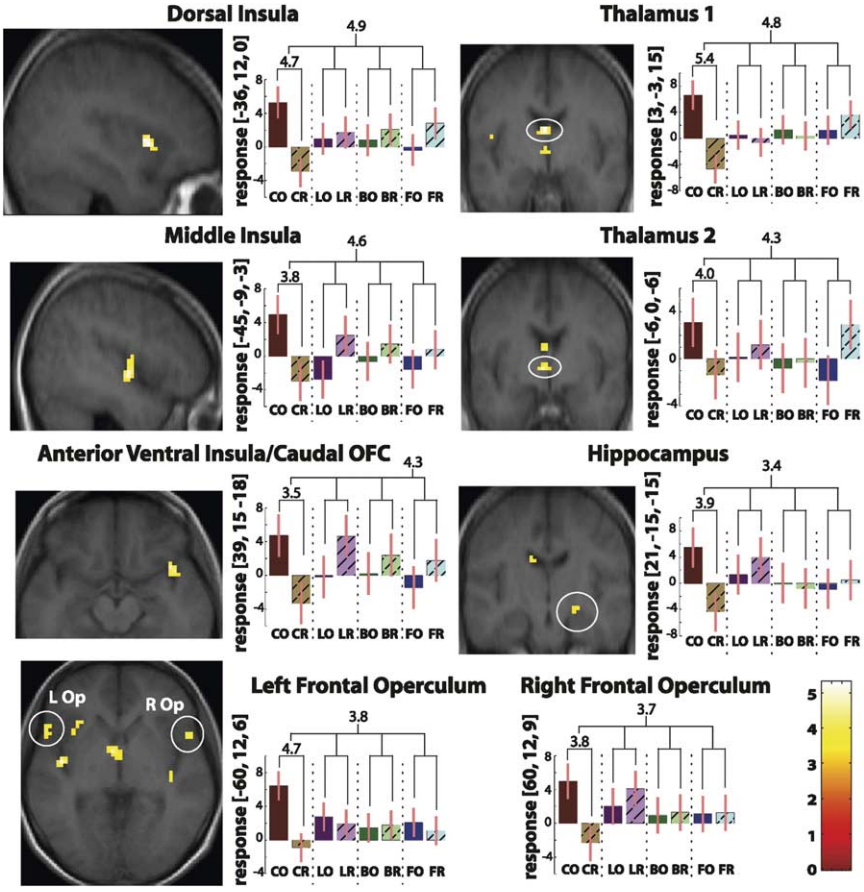

Figure 7. Interaction between Odorant Type and Route of Administration, Showing Regions Responding Preferentially to the Chocolate Odor Delivered Orthonasally

Images from the analysis of {(CO > CR) versus (LO > LR + BO > BR + FO > FR)}. The t map is thresholded at $p < 0.001$ with a cluster threshold of $k < 3$. Graphs show response (parameter estimates) from each of the eight odorant conditions minus each associated baseline condition at the coordinate defined on the y axis. This coordinate corresponds to the activation depicted in the adjacent brain sections (circled if more than one region appears activated). Response is in arbitrary units. (CR = chocolate retro; LR = lavender retro; BR = butanol retro; FR = farnesol retro; CO = chocolate ortho; LO = lavender ortho; BO = butanol ortho; and FO = farnesol ortho). Z values obtained in CO > CR are indicated by the lines adjoining these two conditions, and the z value for the interaction is indicated by the line joining the simple contrasts for the other odors.

Thus, although the finding is suggestive, it represents a weak response, and interpretation is confounded by differences in perceived intensity in three of the four odors. The second, and more striking, demonstration of the effect of route of administration was observed when the response to the chocolate odor was examined alone. This stimulus was perceived as similarly intense and pleasant across both orthonasal and retronasal administration, thus the only perceptual difference was related to where the stimulus was referred (i.e., the nose versus the mouth). Retronasal perception of the chocolate odor led to preferential activation of the perigenual cingulate, medial OFC, posterior cingulate, and superior temporal gyrus, whereas orthonasal perception of this same odor led to preferential activation in several regions of the insula and overlying temporal, parietal, and frontal opercula, hippocampus, caudolateral OFC, thalamus, and amygdala (Figures 6 and 7). Interestingly, this effect did not generalize to three equally pleasant and intense nonfood odors that varied in terms of their physiochemical properties (i.e., lipophilic versus hydrophilic). These results demonstrate that differential neural recruitment during orthonasal versus retronasal olfactory perception may be dependent upon whether an odor has been previously experienced retronasally (i.e., whether it is a food odor).

In 2001, Cerf-Ducastel and Murphy published the first report of neural response to retronasal olfactory stimulation (Cerf-Ducastel and Murphy, 2001). They reported activity in piriform cortex, insula, OFC, hippocampus, and entorhinal cortex in response to aqueous solutions containing an olfactory component. Since these regions have also been shown to respond to orthonasal olfactory perception (Gottfried et al., 2002a; Poellinger et al., 2001; Royet et al., 2003; Savic et al., 2000; Zald and Pardo, 1997; Zatorre et al., 1992), they concluded that both orthonasal and retronasal olfaction rely upon similar neural circuits. However, no direct comparisons were made between orthonasal and retronasal olfactory stimulation. In a subsequent study, de Araujo and

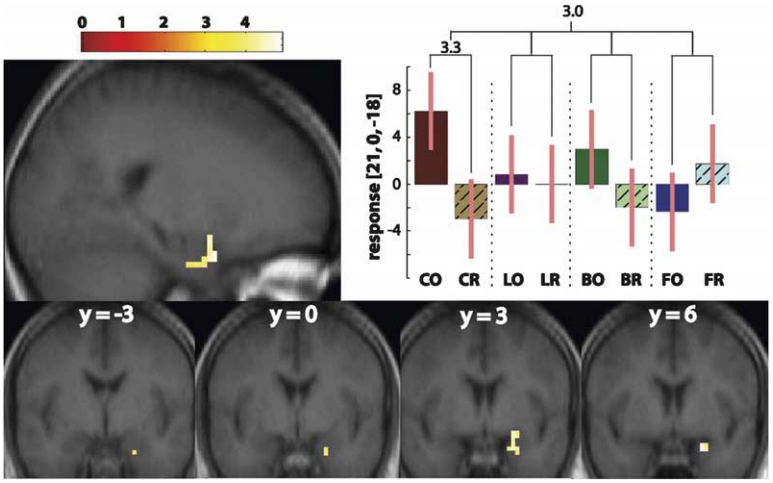

Figure 8. Results from the Region of Interest Analysis Drawn around the Amygdala and Overlying Piriform Cortex Bilaterally for the Contrast CO > All Other Odor Conditions

The t map is thresholded at $p < 0.001$ with a cluster threshold of $k < 3$. Graphs show response (parameter estimates) from each of the eight odorant conditions minus each associated baseline condition at the coordinate defined on the y axis. Response is in arbitrary units. (CR = chocolate retro; LR = lavender retro; BR = butanol retro; FR = farnesol retro; CO = chocolate ortho; LO = lavender ortho; BO = butanol ortho; and FO = farnesol ortho). Z values obtained in CO > CR are indicated by the lines adjoining these two conditions, and the z value for the interaction is indicated by the line joining the simple contrasts for the other odors.

colleagues probed for similarities between orthonasal stimulation in one group of subjects and retronasal stimulation in another group and identified a region of the anterior ventral insula that responded to both routes of olfactory stimulation (de Araujo et al., 2003). Neither of these studies was able to provide a direct comparison between neural recruitment during orthonasal compared to retronasal stimulation because orthonasal stimuli were delivered as vapors and retronasal stimuli, as liquids, resulting in confounds related to stimulus delivery such as mouth movement, swallowing, and sniffing. The current results are consistent with these earlier findings with respect to the many chemosensory regions that respond to odors irrespective of the route of administration. However, our results also extend these findings by demonstrating clear differences in neural response to the same odor when it is delivered in the same phase (e.g., gaseous) and perceived as similarly intense and pleasant by the same group of subjects, but reaches the olfactory epithelium via different routes.

The differential activity observed in response to ortho- versus retronasal delivery of the chocolate odor also provides strong evidence against the notion that retronasal and orthonasal olfaction differ only in the efficiency by which odors are delivered to the olfactory epithelium (Pierce and Halpern, 1996; Voirol and Dagnet, 1986). Similarly, although intensity perception tends to be higher and thresholds lower during orthonasal perception of some odors, the present results confirm that this is not true for all odors (Heilmann and Hummel, 2004). Additionally, there are a handful of recent studies suggesting that the two modes of odor delivery may interact differentially with neural circuits involved in flavor perception and feeding. Slotnick and colleagues have shown that odors can potentiate a taste aversion only when they are presented retronasally (Slotnick et al., 1997), thus implying that retronasal odors have a greater ability to influence the gustatory or flavor neural code. Similarly, in comparison with unimodal taste or smell stimulation, brain response to simultaneously sensed taste and smell is decreased when the odor is delivered orthonasally (Small et al., 1997), but is enhanced when it is delivered retronasally (Small et al., 2004). The current findings provide further support for this hypothesis by indicating that differential activation observed in response to retronasal versus orthonasal perception is influenced by whether an odor represents a food item. However, this interpretation is made cautiously, as further studies are needed to confirm that the effect generalizes to all food odors.

An important question raised by the present finding is why this should be the case. One possibility is that the effect is related to associative learning, since food odors are experienced retronasally in association with oral somatosensory and gustatory stimulation during eating. A second possibility, which is consistent with the pattern of neural responses observed, is that olfactory referral to the mouth may create different reward contexts for food compared to nonfood odors. For example, it is widely acknowledged that reward processing is multifaceted (Bindra, 1978; Dickinson and Balleine, 1994; Kelley and Berridge, 2002; Robbins and Everitt, 1996; Schultz, 2000; White, 1989; Wise, 1985, 2002), and recent neuroimaging studies of monetary reward clearly show distinct neural circuits respond to anticipation versus receipt of monetary reward (Breiter et al., 2001; Knutson et al., 2001, 2003). A similar distinction has been made with respect to food reward. Berridge proposed that food reward is comprised of two components, one associated with the incentive salience of objects predicting food reward, termed "wanting," and one associated with the hedonic pleasure of eating a food reward, termed "liking" (Berridge, 1996). Berridge further speculated that these components arise from distributed neural systems, which may overlap but are clearly separable (Berridge, 1996).

Interpreted within this framework, our results indicate a dissociation in the brain response to a chocolate odor, depending upon whether it is sensed orthonasally and localized to the nose, thus signifying the availability of a food reward, or if it is sensed retronasally and localized to the mouth, thus signifying the receipt of a food reward. Specifically, the insula, opercula, thalamus, hippocampus, amygdala/piriform, and caudolateral OFC respond preferentially to orthonasally sensed chocolate

odor (Figures 7 and 8), whereas the perigenual cingulate, posterior cingulate, medial OFC, and superior temporal gyrus extending into the temporal operculum respond preferentially to chocolate odor sensed retronasally (Figure 6). A similar dissociation was not observed for the nonfood odors, arguably because these odors do not signify availability or receipt of food.

Human neuroimaging studies have shown that the amygdala responds to taste (O'Doherty et al., 2001; Small et al., 2003; Zald et al., 2002, 1998), smell (Anderson et al., 2003; Poellinger et al., 2001; Rolls et al., 2003; Royet et al., 2003, 2000; Zald and Pardo, 1997), flavor (de Araujo et al., 2003; Small et al., 1997), and to the sight of visual cues predicting food reward (Gottfried et al., 2003; Kilgore et al., 2003; LaBar et al., 2001; Morris and Dolan, 2001; O'Doherty et al., 2003). One factor that appears to be important in predicting the magnitude of the amygdala's response to a chemosensory stimulus is its saliency. For example, responses in the amygdala increase with affective significance (Gottfried et al., 2002b; O'Doherty et al., 2001; Rolls et al., 2003; Royet et al., 2003; Zald, 2003; Zald et al., 1998; Zald and Pardo, 1997) and/or intensity (Anderson et al., 2003; Small et al., 2003). However, in the current study, orthonasal and retronasal delivery of the chocolate odor were associated with equivalently intense and pleasant perceptions, yet preferential amygdalar response was observed with the orthonasal stimulation. Thus, in this case, stimulus saliency cannot readily account for the difference in amygdalar activation. Therefore, to account for this finding, we propose that under some circumstances the amygdala is preferentially responsive to sensory cues that provide information about potential reward, rather than received reward. Specifically, we propose that orthonasal sensation of the chocolate odor preferentially engaged the amygdala because, under normal circumstances, orthonasal sensation indicates food availability, whereas retronasal sensation, which is referred to the mouth, normally indicates that a food is being eaten. This can be interpreted as differential responsiveness to food wanting compared to food liking (Berridge, 1996), consistent with work in rodents demonstrating a role for the amygdala in stimulus-reward learning (Everitt et al., 2003; Gallagher, 2000), cue-induced food consumption (Holland et al., 2002; Petrovich et al., 2002) and predicting the reward outcomes of olfactory cues (Schoenbaum et al., 1998, 2003). It is also consistent with work in humans showing that the amygdala responds preferentially to anticipation in comparison with receipt of a pleasant taste (O'Doherty et al., 2002) and with work showing that amygdalar activation does not correlate with changes in subjective ratings of the pleasantness of the taste of a food as it is eaten to satiety (Kringelbach et al., 2003; Small et al., 2001), but that it is sensitive to changes in the reward value (i.e., incentive salience) of stimuli predicting food reward (Gottfried et al., 2003; LaBar et al., 2001; Morris and Dolan, 2001). Thus, it is arguable that the amygdala might, under some circumstances, be preferentially concerned with encoding sensations that provide information about anticipated versus received rewards. An alternative possibility is that anticipated rewards have more "saliency" than received rewards.

Our findings also suggest that the coding and processing of the orthonasally sensed chocolate odor involves the integration of neural processing within a larger network including, in addition to the amygdala/piriform, the frontal operculum, insula, thalamus, caudolateral OFC, and hippocampus. Work in humans (Berns et al., 2001; Gottfried et al., 2003; O'Doherty et al., 2003, 2002) and in animals (Schultz et al., 1998, 2000; Tremblay and Schultz, 1999, 2000) clearly indicates a role for the OFC in prediction of food reward, and the interaction between the amygdala and OFC may be particularly important in encoding the predictive value of cues (Baxter et al., 2000; Gottfried et al., 2003; Schoenbaum et al., 2003). Further, lesions of the insular cortex in rats have been shown to disrupt memory for the incentive value of behavioral outcomes predicting food reward (Balleine and Dickinson, 2000). Anatomical studies showing greater connections between the lateral OFC, amygdala, and insula compared to the medial OFC, amygdala, and insula (Carmichael et al., 1994; Carmichael and Price, 1995a, 1995b, 1996; Morecraft et al., 1992) support the functional coherence of these structures.

A second network was identified that responded more when chocolate was perceived retronasally, presumably indicating that a food reward has been received. This network included the superior temporal gyrus extending into the temporal operculum, perigenual cingulate at the border between Brodmann areas 32 and 25, posterior cingulate cortex, and medial OFC (gyrus rectus). Neuroanatomical investigations have revealed a significant degree of connectivity among these regions. Using autoradiography in the monkey, Pandya and colleagues showed efferent projections from this region of cingulate cortex to the gyrus rectus and superior temporal gyrus (Pandya et al., 1981). Reciprocal connections also exist between the medial OFC and the temporal operculum (Morecraft et al., 1992). In humans, functional connectivity between this region of subcallosal cingulate cortex and the medial OFC has also been demonstrated (Koski and Paus, 2000).

Support for a role for these regions in encoding the receipt of food reward and food liking comes from Small and colleagues, who demonstrated a very strong correlation between decreases in the pleasantness of chocolate as it is eaten beyond satiety and decreases in activity in a region that encompassed the perigenual and subcallosal cingulate, medial OFC, and hypothalamus (Small et al., 2001). Similarly, Kringelbach and colleagues reported a relationship between medial OFC activity and decreases in the pleasantness of chocolate milk and tomato juice after they had been consumed to satiety (Kringelbach et al., 2003). Our finding also accords with data showing that the anterior medial OFC is preferentially responsive to receipt compared with anticipation of a sweet taste (O'Doherty et al., 2002) and with two studies by Knutson and colleagues demonstrating that the ventral medial prefrontal region (overlapping the perigenual and medial OFC regions reported here) is selectively responsive to receiving rather than anticipating monetary reward (Knutson et al., 2001, 2003).

Finally, it is noteworthy that the same chocolate odor activated two distinct regions of OFC depending upon

whether it was perceived ortho- or retronasally. There are multiple reports of functional dissociations of OFC response in the literature and several reviews on the subject (Elliott et al., 2000b; Kringelbach and Rolls, 2004). We have proposed that the medial OFC is more responsive to pleasant stimuli and the lateral OFC is more responsive to unpleasant stimuli (Small et al., 2001). Elliot, Dolan, and Frith suggested that the lateral OFC is more likely to be recruited when a selected action requires suppression of previously rewarded responses (Elliott et al., 2000a), and, more recently, Kringelbach and Rolls postulated an anterior-posterior dissociation. The latter hypothesis suggests that more complex or abstract reinforcers are represented anteriorly, and simpler reinforcers such as taste and pain are represented posteriorly (Kringelbach and Rolls, 2004). Another factor that appears critical in determining OFC activation is stimulus predictability (Berns et al., 2001). However, while there is clearly evidence to support a role for valence, inhibition, complexity-simplicity, and predictability as contributing factors to the selectivity of OFC responsiveness to rewards, none of these factors can account for the rather anterior medial and caudal lateral OFC activations reported here.

An intriguing question remains: By what mechanism is this differential neural activation accomplished? In Rozin's original paper he proposed three possibilities: first, there may be a gating mechanism triggered either by the presence of a palpable substance in the mouth or by the direction of movement of odorants across the olfactory mucosa; second, olfactory input may be combined with available oral inputs into an emergent percept in which the olfactory component loses its identity; and third, the input to the olfactory mucosa may be different under the two conditions. The current results suggest that the presence of a palpable substance in the mouth is not required either to trigger a gating mechanism or to promote the transformation of an olfactory perception into an emergent multimodal flavor percept, since oral stimulation did not differ under the two conditions. Our results also suggest that qualitative differences in the physical stimulus are unlikely to be critical, since the same odorant was presented directly to the space below the mucosa. This leaves direction of flow as a potential mechanism. Importantly, contributions from the respiratory cycle (i.e., the effect of breathing out versus that of breathing in) cannot account for the observed differences because all subjects were performing velopharyngeal closure during the experiment (Kobal, 1981), which prevents airflow from entering the nasal cavity.

The idea that the nature of odorant absorption across the mucosa may contribute important information to olfactory coding was first proposed by Max Mozell (Mozell, 1966; Mozell and Jagodowicz, 1973; Mozell et al., 1969). Interestingly, Kent, Mozell, Youngentob, and Yurco have recently used optical imaging in combination with an olfactory discrimination task in rats to show that discrimination is predicted by odorant-induced mucosal activity patterns (Kent et al., 2003). Although it is clear that subjects localize odorants delivered via the orthonasal tube to the external world and odorants delivered via the retronasal tube to the back of the mouth or throat (Hummel et al., 2005), future studies will be needed to determine if direction-dependent mucosal activity patterns predict this perception.

Conclusions

The main finding of this experiment is that the same odor may produce differential brain responses depending on whether it is sensed orthonasally and experienced as coming from the nose, or is sensed retronasally and experienced as coming from the back of the mouth. This result supports Rozin's hypothesis that orthonasal and retronasal olfaction represent qualitatively distinct sensory experiences. Additionally, the effect of route of delivery was greatest for the chocolate odor, raising the possibility that odorant administration interacts with experience to engage unique brain regions and that olfactory referral induced by retronasal stimulation creates a differential reward context for food but not for nonfood odors by signaling availability versus receipt of food. This hypothesis is consistent with the particular pattern of differential activity in reward circuitry that was observed in response to orthonasal versus retronasal delivery of the chocolate odor. In contrast, the lipophilicity or hydrophilicity of an odorant appears to have no prominent effect upon whether differential responses will be observed to orthonasal versus retronasal olfactory stimulation. Because the current study tested only one food, future experiments are needed to determine whether other food odors produce the same differential brain activations.

Experimental Procedures

Subjects

Eleven healthy right-handed subjects with no known olfactory or gustatory deficits participated in this study. The study was conducted according to the Declaration of Helsinki and was approved by the local ethics committee; subjects provided written consent after being informed about the aims and potential risks of the study. Subjects were instructed not to eat or drink anything for at least 1 hr before the study and reported being neither hungry nor full. Subjects participated in two fMRI sessions. Two odors were presented in each. Odorant order and combination were counterbalanced across days and subjects.

Stimuli and Delivery Apparatus

Four different odors were used: butanol (Merck, Darmstadt, Germany), farnesol (Sigma, Deisenhofen, Germany), lavender (Bell Flavors & Fragrances, Leipzig, Germany), and chocolate (Bell Flavors & Fragrances, Leipzig, Germany). The odors were chosen because all of them were pleasant and had sufficient volatility to be delivered using air dilution olfactometry. Subjects rated these odors for intensity and pleasantness (Figure 2). The number of odorants used was limited by our odor delivery system. A dual olfactometer (OM6b; Burghart Instruments, Wedel, Germany) was employed. One olfactometer delivered odors orthonasally and the other, retronasally so that constant airflows were maintained through both tubes and the subject had no external cue as to where the stimuli had been administered.

Imaging Procedure

The paradigm conformed to a 30 s "ON" 30 s "OFF" block design. Odorants were delivered as 1 s air pulses embedded in a constant airflow (total flow, 1.5 l/min; relative humidity, 80%) throughout the ON period (3 s interstimulus intervals). Odorless air was pulsed in the same fashion during the OFF periods. In half of the blocks, the pulses were delivered retronasally and in the other half of the blocks, orthonasally. A single odorant was used per experimental run. Both the sequence of odorants tested and the site of stimulus

presentation (orthonasal or retronasal) were randomized across subjects. Stimuli were administered nonsynchronously to breathing; the technique of velopharyngeal closure was used to restrict breathing to the mouth (Kobal, 1981). Prior to the experiment, subjects were trained to perform velopharyngeal closure using biofeedback. A thermistor was held in front of the nostril so that subjects were able to see changes in respiratory airflow on an oscilloscope. This element of the experimental design is important because it allows us to rule out contributions of the respiratory cycle. Prior to the fMRI session, subjects were trained on the use of the visual analog scales. Stimulus ratings were collected after each run.

fMRI data were acquired using a gradient echo single shot EPI sequence (T2*-weighted, with TE/TR/bandwidth/flip angle = 40 ms/2.44 s/2605 kHz/90°), which was performed to image the Blood Oxygen Level Dependant (BOLD) effect. Twenty-six slices were acquired (3 mm thickness; 0.75 mm gap; field-of-view, 192 mm; matrix, 64 × 64) that covered the brain and were oriented parallel to the cribriform plate to minimize bone artifacts. In each functional run, 120 volumes (plus three volumes at the beginning, to equilibrate magnetization) were collected, resulting in a scan time of approximately 5 min per run. A complementary T1-weighted, high-resolution structural image set was acquired using a 3D sequence.

Data Analysis

Neuroimaging data were pre- and postprocessed with SPM2. (Wellcome Department of Cognitive Neurology, London, UK, implemented in MATLAB 6.5 R13, The MathWorks, Inc., Natick, MA). Functional data were registered, motion corrected, and resliced with use of the preprocessing procedures of SPM. The resulting images were coregistered to its corresponding T1 volume. Analyses were carried out on spatially normalized (stereotactically transformed into MNI-space; MNI-template supplied with SPM2) and smoothed images (a 7 mm full width at half maximum [FWHM] Gaussian kernel for individual analyses and a 10 mm FWHM Gaussian kernel for the group analysis).

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